abcam

Product datasheet

Anti-Alpha-synuclein antibody [EPR20535] - BSA and Azide free ab225866

KO VALIDATED Recombinant RabMAb

12 Images

Overview

Product name	Anti-Alpha-synuclein antibody [EPR20535] - BSA and Azide free
Description	Rabbit monoclonal [EPR20535] to Alpha-synuclein - BSA and Azide free
Host species	Rabbit
Tested applications	**Suitable for:** WB, IHC-P, IHC-Fr, IP
Species reactivity	**Reacts with:** Mouse, Rat, Human, Recombinant fragment
Immunogen	Recombinant full length protein. This information is proprietary to Abcam and/or its suppliers.
Positive control	IHC-P: Human cerebral cortex tissue.
General notes	ab225866 is the carrier-free version of **ab212184**. Our **carrier-free** antibodies are typically supplied in a PBS-only formulation, purified and free of BSA, sodium azide and glycerol. The carrier-free buffer and high concentration allow for increased conjugation efficiency. This conjugation-ready format is designed for use with fluorochromes, metal isotopes, oligonucleotides, and enzymes, which makes them ideal for antibody labelling, functional and cell-based assays, flow-based assays (e.g. mass cytometry) and Multiplex Imaging applications. Use our **conjugation kits** for antibody conjugates that are ready-to-use in as little as 20 minutes with <1 minute hands-on-time and 100% antibody recovery: available for fluorescent dyes, HRP, biotin and gold. This product is compatible with the Maxpar® Antibody Labeling Kit from Fluidigm, without the need for antibody preparation. Maxpar® is a trademark of Fluidigm Canada Inc. Our RabMAb® technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to **RabMAb® patents**.

Properties

Form	Liquid
Storage instructions	Shipped at 4°C. Store at +4°C. Do Not Freeze.
Storage buffer	pH: 7.2

	Constituent: PBS
Carrier free	Yes
Purity	Protein A purified
Clonality	Monoclonal
Clone number	EPR20535
Isotype	IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab225866 in the following tested applications.

The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

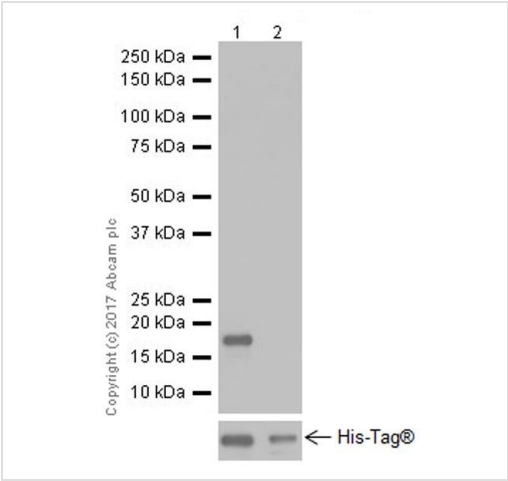
Application	Abreviews	Notes
WB		Use at an assay dependent concentration. Detects a band of approximately 18 kDa (predicted molecular weight: 14 kDa).
IHC-P		Use at an assay dependent concentration. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.
IHC-Fr		Use at an assay dependent concentration. Antigen retrieval: Heated citrate solution (10mM citrate pH 6.0 + 0.05% Tween-20).
IP		Use at an assay dependent concentration.

Target

Function	May be involved in the regulation of dopamine release and transport. Induces fibrillization of microtubule-associated protein tau. Reduces neuronal responsiveness to various apoptotic stimuli, leading to a decreased caspase-3 activation.
Tissue specificity	Expressed principally in brain but is also expressed in low concentrations in all tissues examined except in liver. Concentrated in presynaptic nerve terminals.
Involvement in disease	Genetic alterations of SNCA resulting in aberrant polymerization into fibrils, are associated with several neurodegenerative diseases (synucleinopathies). SNCA fibrillar aggregates represent the major non A-beta component of Alzheimer disease amyloid plaque, and a major component of Lewy body inclusions. They are also found within Lewy body (LB)-like intraneuronal inclusions, glial inclusions and axonal spheroids in neurodegeneration with brain iron accumulation type 1. Parkinson disease 1 Parkinson disease 4 Dementia Lewy body
Sequence similarities	Belongs to the synuclein family.
Domain	The 'non A-beta component of Alzheimer disease amyloid plaque' domain (NAC domain) is involved in fibrils formation. The middle hydrophobic region forms the core of the filaments. The C-terminus may regulate aggregation and determine the diameter of the filaments.
Post-translational	Phosphorylated, predominantly on serine residues. Phosphorylation by CK1 appears to occur on

modifications	residues distinct from the residue phosphorylated by other kinases. Phosphorylation of Ser-129 is selective and extensive in synucleinopathy lesions. In vitro, phosphorylation at Ser-129 promoted insoluble fibril formation. Phosphorylated on Tyr-125 by a PTK2B-dependent pathway upon osmotic stress. Hallmark lesions of neurodegenerative synucleinopathies contain alpha-synuclein that is modified by nitration of tyrosine residues and possibly by dityrosine cross-linking to generated stable oligomers. Ubiquitinated. The predominant conjugate is the diubiquitinated form. Acetylation at Met-1 seems to be important for proper folding and native oligomeric structure.
Cellular localization	Cytoplasm, cytosol. Membrane. Nucleus. Cell junction, synapse. Secreted. Membrane-bound in dopaminergic neurons.

Images



Western blot - Anti-Alpha-synuclein antibody [EPR20535] - BSA and Azide free (ab225866)

All lanes : Anti-Alpha-synuclein antibody [EPR20535] (**ab212184**) at 1/1000 dilution

Lane 1 : Human Alpha-synuclein recombinant protein
Lane 2 : Human Beta-synuclein recombinant protein

Lysates/proteins at 0.01 µg per lane.

Secondary
All lanes : Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/100000 dilution

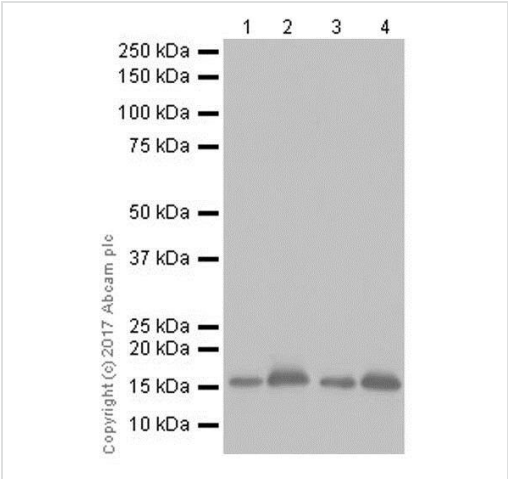
Predicted band size: 14 kDa
Observed band size: 18 kDa

Exposure time: 8 seconds

This data was developed using **ab212184**, the same antibody clone in a different buffer formulation

Blocking/Dilution buffer: 5% NFDM/TBST.

Human Alpha-synuclein recombinant protein contain aa1-140 with His-tag. Human Beta-synuclein recombinant protein contain aa1-134 with His-tag.



Western blot - Anti-Alpha-synuclein antibody [EPR20535] - BSA and Azide free (ab225866)

All lanes : Anti-Alpha-synuclein antibody [EPR20535] (**ab212184**) at 1/1000 dilution

Lane 1 : Human cerebellum lysate
Lane 2 : Human brain lysate
Lane 3 : Mouse brain lysate
Lane 4 : Rat brain lysate

Lysates/proteins at 20 µg per lane.

Secondary
All lanes : Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/100000 dilution

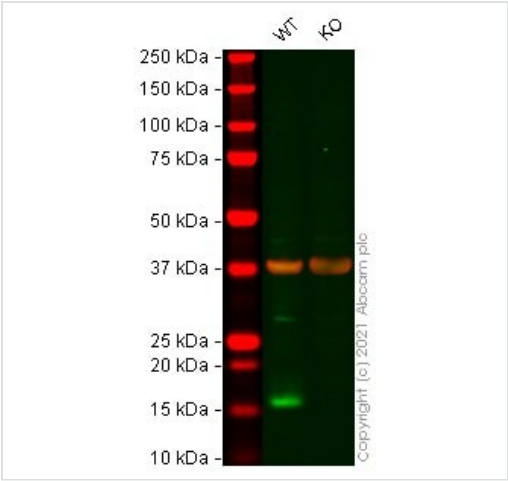
Predicted band size: 14 kDa
Observed band size: 18 kDa

Exposure time: 5 seconds

This data was developed using **ab212184**, the same antibody clone in a different buffer formulation

Blocking/Dilution buffer: 5% NFDM/TBST.

The observed molecular weight is consistent with the literature (PMID: 11739566).



Western blot - Anti-Alpha-synuclein antibody [EPR20535] - BSA and Azide free (ab225866)

All lanes : Anti-Alpha-synuclein antibody [EPR20535] (**ab212184**) at 1/1000 dilution

Lane 1 : Wild-type U-87 MG cell lysate
Lane 2 : SNCA knockout U-87 MG cell lysate

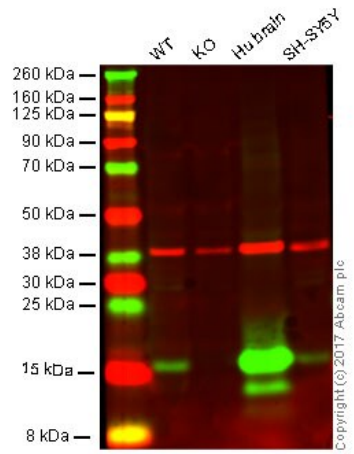
Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

Predicted band size: 14 kDa
Observed band size: 16 kDa

This data was developed using **ab212184**, the same antibody clone in a different buffer formulation:

False colour image of Western blot: Anti-Alpha-synuclein antibody [EPR20535] staining at 1/1000 dilution, shown in green; Mouse anti-GAPDH antibody [6C5] (**ab8245**) loading control staining at 1/20000 dilution, shown in red. In Western blot, **ab212184** was shown to bind specifically to Alpha-synuclein. A band was observed at 16 kDa in wild-type U-87 MG cell lysates with no signal observed at this size in SNCA knockout cell line **ab282333** (knockout cell lysate **ab283006**). To generate this image, wild-type and SNCA knockout U-87 MG cell lysates were analysed. First, samples were run on an SDS-PAGE gel then transferred onto a nitrocellulose membrane. Membranes were blocked in 3 % milk in TBS-0.1 % Tween® 20 (TBS-T) before incubation with primary antibodies overnight at 4°C. Blots were washed four times in TBS-T, incubated with secondary antibodies for 1 h at room temperature, washed again four times then imaged. Secondary antibodies used were Goat anti-Rabbit IgG H&L (IRDye® 800CW) preabsorbed (**ab216773**) and Goat anti-Mouse IgG H&L (IRDye® 680RD) preabsorbed (**ab216776**) at 1/20000 dilution.



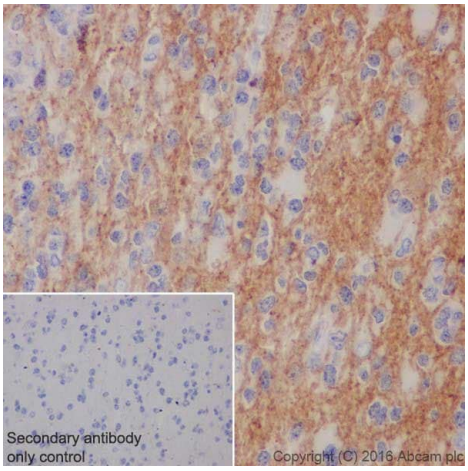
Western blot - Anti-Alpha-synuclein antibody [EPR20535] - BSA and Azide free (ab225866)

This data was developed using **ab212184**, the same antibody clone in a different buffer formulation:

Lane 1: Wild type HAP1 whole cell lysate (20 µg)
Lane 2: SNCA (alpha Synuclein) knockout HAP1 whole cell lysate (20 µg)
Lane 3: Human brain whole tissue lysate (20 µg)
Lane 4: SH-SY5Y whole cell lysate (20 µg)

Lanes 1 - 4: Merged signal (red and green). Green - **ab212184** observed at 14 kDa. Red - loading control, **ab9484**, observed at 37 kDa.

ab212184 was shown to specifically react with SNCA (alpha Synuclein) in wild type cells as signal was lost in SNCA (alpha Synuclein) knockout cells. Wild-type and SNCA (alpha Synuclein) knockout samples were subjected to SDS-PAGE. **ab212184** and **ab9484** (Mouse anti-GAPDH loading control) were incubated overnight at 4°C at a 1/1000 dilution and 1/20000 dilution respectively. Blots were developed with Goat anti-Rabbit IgG H&L (IRDye® 800CW) preabsorbed **ab216773** and Goat anti-Mouse IgG H&L (IRDye® 680RD) preabsorbed **ab216776** secondary antibodies at 1/20000 dilution for 1 hour at room temperature before imaging.



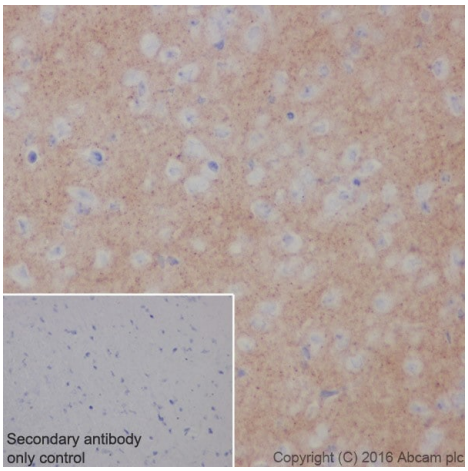
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Alpha-synuclein antibody [EPR20535] - BSA and Azide free (ab225866)

Immunohistochemical analysis of paraffin-embedded human glioma tissue labeling Alpha-synuclein with **ab212184** at 1/16000 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) Ready to use.

Cytoplasmic staining on human glioma [PMID: 22112368].
Counter stained with Hematoxylin.

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit IgG H&L (HRP) Ready to use.
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab212184**).

Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.



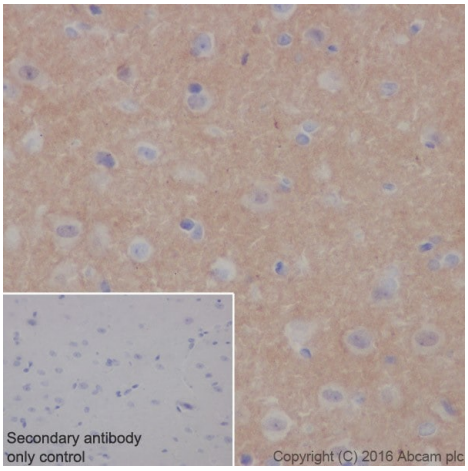
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Alpha-synuclein antibody [EPR20535] - BSA and Azide free (ab225866)

Immunohistochemical analysis of paraffin-embedded mouse cerebral cortex tissue labeling Alpha-synuclein with **ab212184** at 1/16000 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) Ready to use.

Cytoplasmic staining on mouse cerebral cortex [PMID: 22112368].
Counter stained with Hematoxylin.

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit IgG H&L (HRP) Ready to use.
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab212184**).

Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Alpha-synuclein antibody [EPR20535] - BSA and Azide free (ab225866)

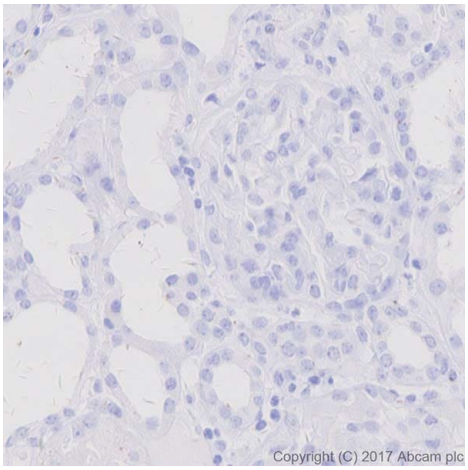
Immunohistochemical analysis of paraffin-embedded rat cerebral cortex tissue labeling Alpha-synuclein with **ab212184** at 1/16000 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) Ready to use.

Cytoplasmic staining on rat cerebral cortex [PMID: 22112368]. Counter stained with Hematoxylin.

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit IgG H&L (HRP) Ready to use.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab212184**).

Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.



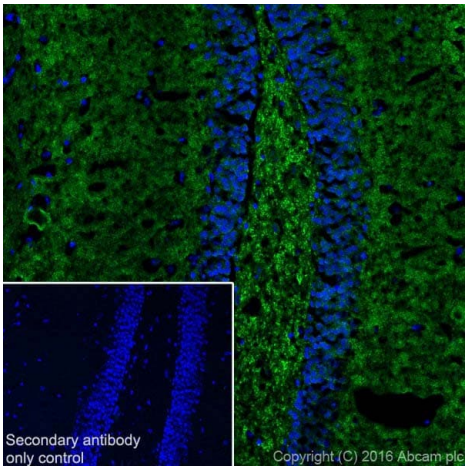
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Alpha-synuclein antibody [EPR20535] - BSA and Azide free (ab225866)

Immunohistochemical analysis of paraffin-embedded human kidney tissue labeling Alpha-synuclein with **ab212184** at 1/16000 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) Ready to use.

Negative control: No staining on human kidney. [PMID: 14997013].
Counter stained with Hematoxylin.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab212184**).

Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.



Immunohistochemistry (Frozen sections) - Anti-Alpha-synuclein antibody [EPR20535] - BSA and Azide free (ab225866)

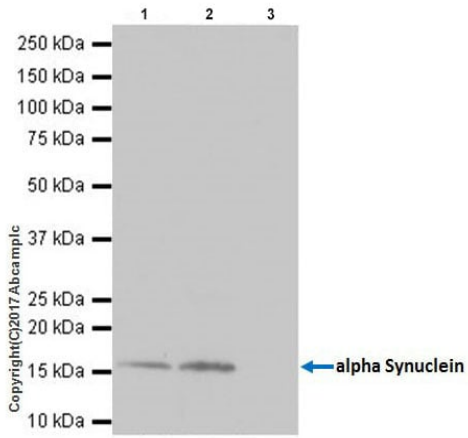
Immunohistochemical analysis of 4% paraformaldehyde-fixed, 0.2% Triton X-100 permeabilized frozen mouse hippocampus tissue labeling Alpha-synuclein with **ab212184** at 1/100 dilution, followed by Goat anti-rabbit IgG (Alexa Fluor® 488) (**ab150077**) secondary antibody at 1/1000 dilution (green).

Cytoplasmic staining on mouse hippocampus (PMID: 22112368).

The nuclear counterstain is DAPI (blue).

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat anti-rabbit IgG (Alexa Fluor® 488) (**ab150077**) at 1/1000 dilution.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab212184**).



Immunoprecipitation - Anti-Alpha-synuclein antibody [EPR20535] - BSA and Azide free (ab225866)

Alpha-synuclein was immunoprecipitated from 0.35 mg of mouse brain lysate with **ab212184** at 1/30 dilution.

Western blot was performed from the immunoprecipitate using **ab212184** at 1/1000 dilution.

VeriBlot for IP Detection Reagent (HRP) (**ab131366**), was used for detection at 1/1000 dilution.

Lane 1: Mouse brainlysate 10ug (Input).

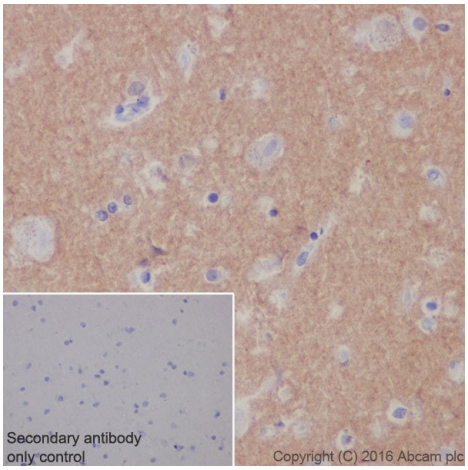
Lane 2: **ab212184** IP in mouse brain lysate.

Lane 3: Rabbit monoclonal IgG (**ab172730**) instead of **ab212184** in mouse brain lysate.

Blocking and dilution buffer and concentration: 5% NFDM/TBST.

Exposure time: 1 second.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab212184**).



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Alpha-synuclein antibody [EPR20535] - BSA and Azide free (ab225866)

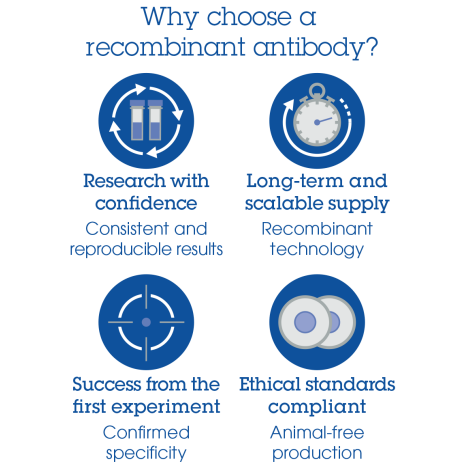
Immunohistochemical analysis of paraffin-embedded human cerebral cortex tissue labeling Alpha-synuclein with **ab212184** at 1/16000 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) Ready to use.

Cytoplasmic staining on human cerebral cortex [PMID: 22112368]. Counter stained with Hematoxylin.

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit IgG H&L (HRP) Ready to use.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab212184**).

Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.



Anti-Alpha-synuclein antibody [EPR20535] - BSA and Azide free (ab225866)

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